Genetic Characteristics of Mismatch Repair-deficient Glioblastoma

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Abstract

Mismatch repair (MMR) gene deficiency is rarely observed in gliomas, a constitutional defect is associated with tumorigenesis in Lynch syndrome, and an acquired defect is associated with hypermutation after temozolomide treatment. However, the meaning of MMR gene deficiency in gliomas is unclear. Two cases of MMR-deficient glioblastomas are reported, and mutational status of oncogenes was compared between primary and recurrent tumor samples in a glioblastoma patient with Lynch syndrome. Additionally, the characteristics of MMR-deficient glioblastomas were analyzed using public glioma datasets to determine the meaning of MMR deficiency in gliomas. Case 1 was a glioblastoma patient with Lynch syndrome, and treatment with pembrolizumab for the recurrent tumor was temporarily effective for a short period. Comparison of mutational changes between primary and recurrent tumor samples showed many additional mutated genes associated with multiple signaling pathways in the recurrent tumor. Tumor recurrence and chemoresistance could be associated with intratumoral heterogeneity and accelerated tumor progression due to defects of multiple signaling pathways. Case 2 was a glioblastoma patient with acquired MMR gene deficiency, and she died of rapid progression of bone marrow metastases. This rare clinical course was considered to be associated with gene expression changes and heterogeneity that resulted from MMR gene deficiency. Two cases of MMR gene-deficient glioblastomas were presented, and their genetic characteristics suggested that their clinical courses could be associated with MMR gene deficiency.

Keywords: mismatch repair, Lynch syndrome, glioma, bone marrow, pembrolizumab

Introduction

Gliomas are malignant brain tumors that are incurable despite surgical resection, radiation therapy, and chemotherapy, and new treatments are needed. Some gliomas develop in patients with Lynch syndrome, which is the most common hereditary cancer syndrome causing colorectal, endometrial, ovarian, gastric, urothelial tract, biliary tract, pancreatic, and prostatic cancers.[1] The oncogenic tendency of Lynch syndrome is derived from a constitutional defect of mismatch repair (MMR) genes including *MSH6*, *PMS2*, *MSH2*, and *MLH1*. An acquired defect of the MMR gene is also observed in some glioma patients treated with alkylating chemotherapy (temozolomide) and is associated with hypermutation and chemoresistance.[2] MMR is a system for recognizing and repairing erroneous insertion, deletion, and misincorporation of bases that can arise during DNA replication and recombination, as well as repairing some forms of DNA damage.[3] A defect of the MMR system leads to accumulation of mutational load and genomic instability, which generates intratumoral heterogeneity resulting in evolutionary change and tumor progression.[4] In contrast, accumulation of mutational load leads to neoantigen load, which is associated with clinical responses to immune

checkpoint therapies.[4] Immunohistochemistry (IHC) for the MMR proteins could be a viable and reliable frontline screening test for MMR-deficient gliomas.[5] We have performed IHC of MMR proteins for gliomas in which immunotherapy was being considered. However, the meaning of MMR gene deficiency in gliomas is unclear. Two cases of MMR-deficient glioblastomas are reported, and mutational changes were compared between primary and recurrent tumor samples in the glioblastoma patient with Lynch syndrome. Additionally, the characteristics of MMR-deficient glioblastomas were analyzed using The Cancer Genome Atlas (TCGA) datasets to determine the meaning of MMR deficiency in gliomas.

Case Report

Case 1

A 79-year-old woman with motor aphasia and agraphia was referred to our hospital because of a brain tumor in the left parietal lobe. She had been treated for ascending colon cancer, and she and her family had been diagnosed with Lynch syndrome by genetic screening (Fig. 1A). Magnetic resonance imaging (MRI) showed a ring-enhancing lesion in the left parietal lobe accompanied by peritumoral edema (Fig. 1B). The Karnofsky performance status (KPS) was 90 before treatment. Gross total resection of the tumor was performed, and it was diagnosed as glioblastoma, *IDH* wild type (Fig. 1H). Immunohistochemical examination showed loss of MSH2 expression, *TP53* mutation, and an MIB-1 index of 47%. MSH6 expression was partially lost accompanied with loss of MSH2 expression (Fig. 1I). *O6-methylguanine-DNA methyltransferase (MGMT)* gene promoter methylation was detected by methylation-specific polymerase chain reaction (PCR). Microsatellite instability (MSI) was high. Local irradiation of 34 Gy concomitant with temozolomide was performed as adjuvant therapy (Fig. 1H). After 10 cycles of maintenance temozolomide therapy, her motor aphasia worsened, and tumor progression was observed on MRI 11 months after the surgery (Fig. 1C). A second resection was performed for recurrence of glioblastoma (Fig. 1D). Pembrolizumab was given as the second-line chemotherapy because of MSI detected in the tumor (Fig. 1E). Partial response was observed on MRI after two cycles of pembrolizumab (Fig. 1F), but the tumor showed progression after three cycles (Fig. 1G). The patient died of tumor progression 15.5 months after the first surgery.

Mutational analysis of the primary tumor and recurrent tumor samples was performed by the Oncomine Tumor Mutation Load Assay (Thermo Fisher Scientific, MA, USA), and their mutational status was compared. Germline nonframeshift deletion of MSH2 gene, p.Leu92del, was detected in both samples. In all, 25 nonsynonymous somatic mutated genes were detected in the primary tumor, and 53 mutated genes were newly added in the recurrent tumor (Supplementary Table 1; the supplementary table is available online). The tumor mutation burden was elevated from 21.6/Mb to 101.6/Mb in the recurrent tumor. The nonsynonymous mutated genes in the primary tumor belonged to a signaling pathway (*PTEN*), the p53 pathway (*TP53*), and the RB1 pathway (*RB1*). In contrast, most of the additional mutated genes in the recurrent tumor belonged to signaling pathways (*PI3K, NF1, KIT, NOTCH, ERBB, FGFR*, and *SMAD*) (Fig. 1J).

Case 2

A 45-year-old woman with left dysesthesia and unilateral spatial neglect was referred to our hospital because of a brain tumor in the right parietal lobe. She had no remarkable past or family history. The KPS was 90 before treatment. MRI showed a ring-enhancing lesion in the right parietal lobe, and gross total resection of the tumor was performed (Fig. 2A and 2B). Histological examination revealed proliferation of highly anaplastic glial cells with nuclear hyperchromasia and pleomorphism. Pseudopalisading necrosis was frequently observed. The diagnosis was glioblastoma, *IDH* wild type. Immunohistochemical examination showed loss of MLH1 expression, *TP53* mutation, and an MIB-1 index of 54%. PMS2 expression was also lost accompanied with loss of MLH1 expression (Fig. 2E). *MGMT* gene promoter methylation was undetected. MSI was low. Local irradiation of 60 Gy concomitant with temozolomide was performed as adjuvant therapy. After two cycles of maintenance temozolomide therapy, tumor progression was observed on MRI 5.3 months after surgery (Fig. 2C). A second resection was performed for recurrence of glioblastoma, and additional local irradiation of 35 Gy was performed for remnant tumor. Lower back pain and thrombocytopenia were observed 20 days after the second surgery, with rapid progression. Spinal MRI showed vertebral metastases. Bone marrow biopsy was performed 7.0 months after the first surgery. Histological examination revealed solid proliferation of the malignant tumor cells with high nucleocytoplasmatic ratio in a little bone marrow. The tumor cells were positive for GFAP, synaptophysin, and OLIG2, indicating that they were metastases of glioblastoma (Fig. 2F). These tumor cells also showed loss of MLH1 and PMS2 expression
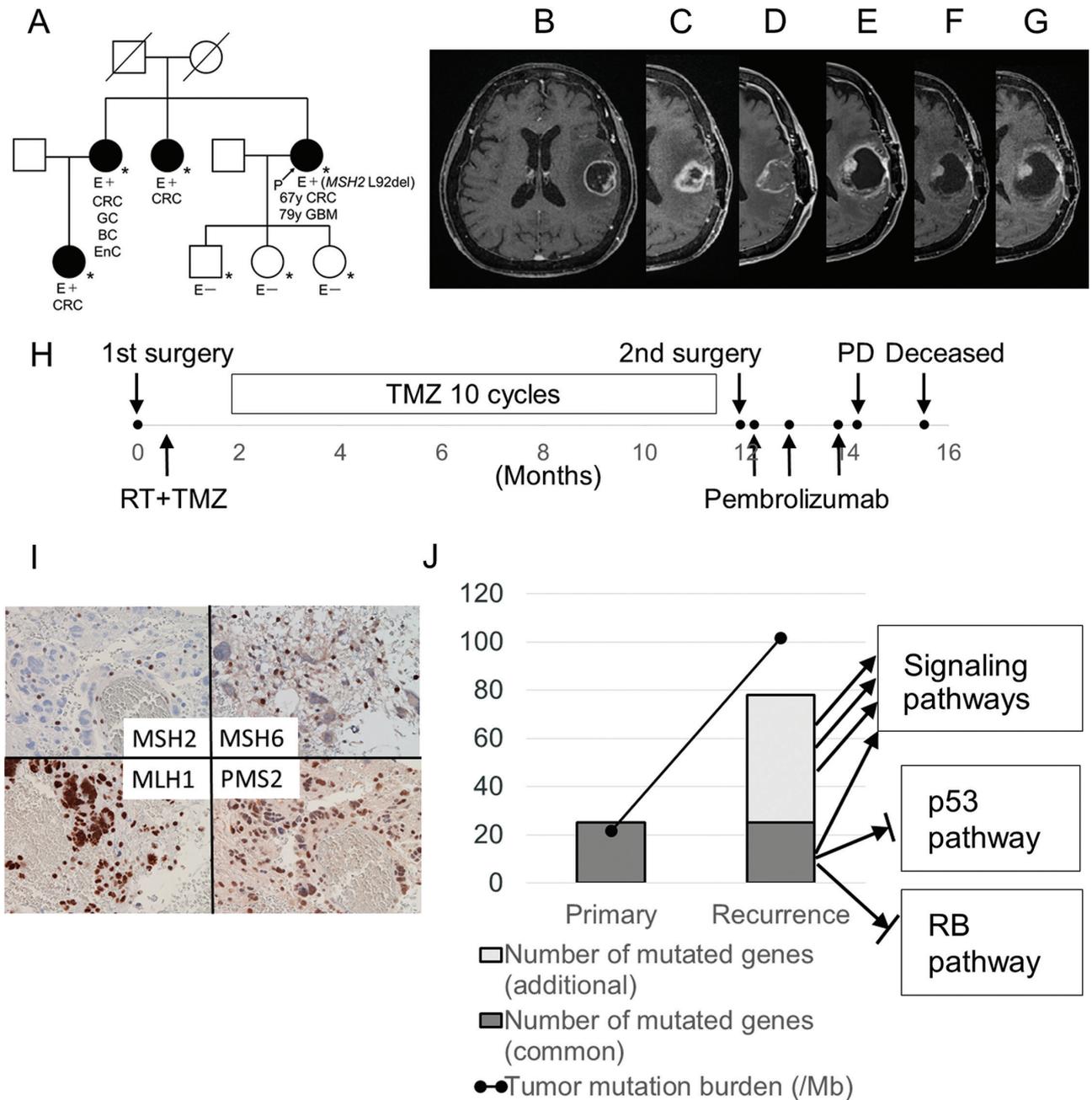
Fig. 1 **(A)** The family tree for Case 1. The squares and circles indicate male and female, respectively. P indicates proband, and filled circles indicate affected. * indicates the patients evaluated by genetic examination. E+ and E- indicate positive and negative for genetic examination of Lynch syndrome, respectively. CRC, GC, BC, EnC, and GBM indicate colorectal cancer, gastric cancer, breast cancer, endometrial cancer, and glioblastoma. **(B–G)** Serial changes of the glioblastoma on gadolinium-enhanced T1-weighted MRI. **(B)** Preoperative image of the primary tumor before the first surgery; **(C)** preoperative image of the recurrent tumor before the second surgery; **(D)** postoperative image of the second surgery; **(E)** the image before treatment with pembrolizumab; **(F)** the image after two cycles of pembrolizumab treatment. **(G)** The image after three cycles of pembrolizumab treatment. **(H)** Time course of treatment for case 1. The base point of the time line is the date of the first surgery. 1st surgery: tumor resection for primary glioblastoma; RT+TMZ: extended local irradiation concomitant with temozolomide therapy; 2nd surgery: tumor resection for recurrent glioblastoma; PD: progressive disease. **(I)** IHC for the MMR proteins; MSH2, MSH6, MLH1, and PMS2. **(J)** Changes of mutated genes and tumor mutation burden compared between primary and recurrent tumors. Mutated genes observed in the primary tumor were associated with a signaling pathway, the p53 pathway, and the RB pathway, but mutated genes added in the recurrent tumor were associated with multiple signaling pathways. IHC: immunohistochemistry, MRI: magnetic resonance imaging.
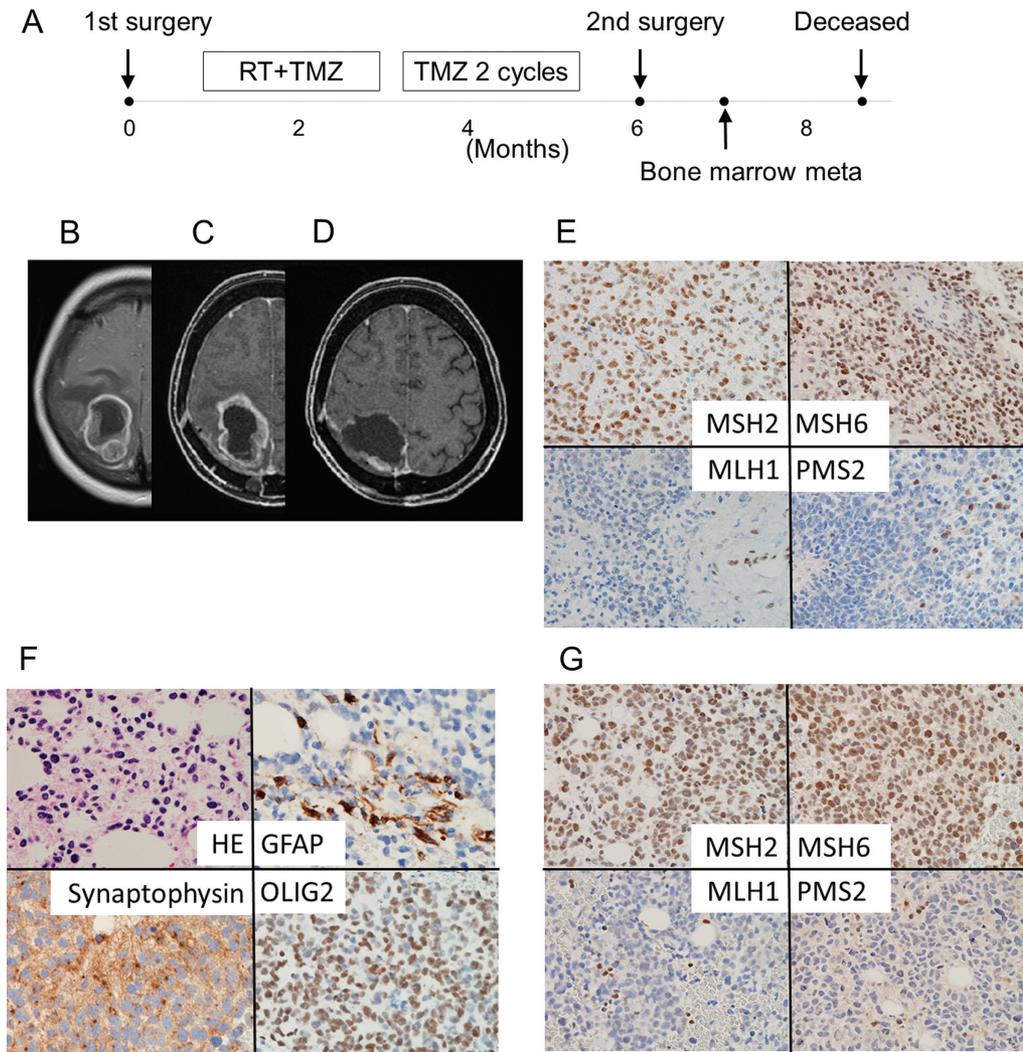
Fig. 2 (A) Time course of treatment for case 2. The base point of the time line is the date of the first surgery. 1st surgery: tumor resection for primary glioblastoma; RT+TMZ: extended local irradiation concomitant with temozolomide therapy; 2nd surgery: tumor resection for recurrent glioblastoma; Bone marrow meta: the date bone marrow biopsy was performed. (B–D) Serial changes of the glioblastoma on gadolinium-enhanced T1-weighted MRI. (B) Preoperative image of the primary tumor before the first surgery; (C) preoperative image of the recurrent tumor before the second surgery; (D) the image on the date bone marrow biopsy was performed. (E) IHC of the primary tumor for the MMR proteins. (F) Pathological findings of the bone marrow. Hematoxylin–eosin staining and immunostaining for GFAP, synaptophysin, and OLG2 are presented. (G) Immunohistochemistry of the bone marrow for the MMR proteins. IHC: immunohistochemistry, MRI: magnetic resonance imaging.

(Fig. 2G). Intracranial tumor progression was not observed at this point (Fig. 2D), but she died of progression of pancytopenia 8.6 months after the first surgery.

MMR-deficient glioma cases in the TCGA dataset

A total of 1274 glioma cases of the TCGA dataset were analyzed using the cBioPortal website (http://www.cbioportal.org/). Of the 1274 analyzed gliomas, 499 cases were *IDH* wild type, 535 were *IDH* mutant, and 240 had no data. MMR gene mutation was observed in 3% of *IDH* wild type and in 2% of *IDH* mutant gliomas. Of the MMR gene mutations, *MSH6* mutation accounted for 43%. Mutations of *PMS2*, *MLH1*, and *MSH2* were detected in 17%, 14%, and 12% of cases, respectively, and mutations of multiple MMR genes were detected in 14%. Additionally, *MSH6* mutation was observed in all cases with multiple mutations. MMR gene mutation was observed significantly more frequently in recurrent tumors than in primary tumors (13% vs. 1%, $p = 0.001$, Fisher's exact test). The number of nonsynonymous mutated genes was significantly higher in MMR gene mutant gliomas than in MMR gene wild-type
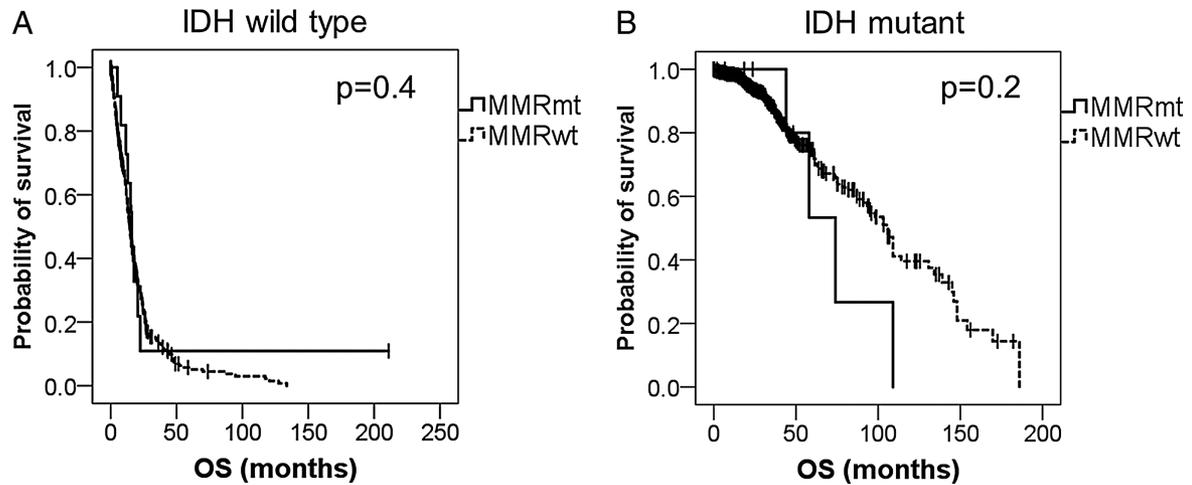
Fig. 3 Kaplan–Meier curves of overall survival in the *IDH* wild type (A) and *IDH* mutant gliomas (B) comparing between the MMR gene mutant group and the MMR gene wild-type group are presented. There is no difference between the MMR mutant group and the MMR wild-type group, both in *IDH* wild-type gliomas and in *IDH* mutant gliomas (p = 0.4 in *IDH* wild type, p = 0.2 in *IDH* mutant). MMR: mismatch repair, MMR mt: MMR gene mutant group, MMR wt: MMR gene wild-type group.

Table 1 Mutated genes frequently observed in MMR mutant gliomas compared with MMR wild type gliomas

Gene	MMR mutant	MMR wild-type	p value
IDH wild type			
NF1	47%	8%	<0.01
TP53	52%	20%	<0.01
EP300	21%	0%	<0.01
GATA3	21%	0%	<0.01
NOTCH1	21%	0%	<0.01
IDH mutant			
TP53	92%	59%	<0.01
NOTCH2	58%	1%	<0.01
FAT1	50%	2%	<0.01

MMR mutant: mismatch repair gene mutant group, MMR wild-type: mismatch repair gene wild-type group.

gliomas (p <0.0001, student's t-test). Overall survival of the MMR mutant glioma patients was no different from that of the MMR wild-type glioma patients, both in *IDH* mutant and *IDH* wild-type gliomas (p = 0.4 in *IDH* wild type, Fig. 3A, p = 0.2 in *IDH* mutant, Fig. 3B, Log-rank test). The mutated genes frequently observed in MMR mutant gliomas compared with MMR wild-type gliomas were *NF1, TP53, EP300, GATA3*, and *NOTCH1* gene in *IDH* wild-type gliomas, and *TP53, NOTCH2*, and *FAT1* in *IDH* mutant gliomas (Table 1). Comparing expression levels of over 20000 genes between two cases of MMR mutant gliomas and ten cases of MMR

wild-type gliomas in *IDH* wild-type gliomas showed that 3116 genes were significantly upregulated, and 143 genes were significantly downregulated in MMR mutant gliomas. Upregulated genes belonged to the TNF signaling pathway associated with tumor progression, and downregulated genes belonged to the Rap1 signaling pathway associated with cell adhesion.

Discussion

We reported two cases of MMR-deficient glioblastomas showing characteristic clinical course; case 1 showed effectiveness of pembrolizumab and case 2 showed bone marrow metastases resulting in poor prognosis.

Increased expression of neoantigens promoted the effectiveness of immune checkpoint inhibitors for MMR-deficient cancers,[6] but effectiveness for gliomas was limited because of the intratumoral heterogeneity of MMR deficiency.[5,7,8] Heterogeneity of MMR deficiency was resulted from acquired MMR gene mutation observed in gliomas treated with temozolomide. Effectiveness of pembrolizumab observed in case 1 could be a result from homogeneous MMR deficiency by germ line mutation of MMR gene. Further analysis is needed to evaluate the effectiveness of immune checkpoint inhibitors for MMR-deficient gliomas with germ line mutation of MMR genes.

The tumor mutation burden of Case 1 was high and rapidly elevated at recurrence, as expected. Mutated genes observed in glioblastomas belong to any one of three pathways: signaling pathways, p53

pathways, and RB pathways.[9] Mutation genes observed in the primary tumor of case 1 belonged to all three pathways, suggesting that defects of all three pathways were important for tumorigenesis. In contrast, the characteristics of additional mutated genes observed in recurrent tumor indicated that defects of multiple signaling pathways were associated with tumor recurrence by developing intra-tumoral heterogeneity, accelerating tumor progression, and promoting chemoresistance. High intratumoral heterogeneity could be associated with the short period that pembrolizumab was effective for the recurrent tumor.

The MMR-deficient glioblastoma of case 2 showed bone marrow metastases, which are very rare in glioblastomas; only two cases were reported in the last 20 years.[10,11] MMR deficiency was reported to promote brain metastasis in colon cancer due to induced intratumoral heterogeneity.[12] The bone marrow metastases could have been derived from MMR deficiency in case 2. Poor effectiveness of temozolomide observed in case 2 could be a result from MMR deficiency in addition to *MGMT* gene promoter unmethylation. MMR deficiency and *MGMT* gene promoter unmethylation was associated with resistance to temozolomide in glioblastoma,[13,14] but Poly (ADP-ribose) polymerase (PARP) inhibitors could re-sensitize to temozolomide.[15] PARP inhibitors could be a therapeutic option for case 2. The MMR deficiency of glioblastoma in case 2 was considered to be acquired deficiency because (1) she had no remarkable past or family history and (2) MSI of the tumor was low. Single cell analysis revealed that intratumoral heterogeneity of MMR deficiency resulted in low MSI in the acquired MMR-deficient glioblastoma.[7]

The frequency of MMR gene mutations in gliomas was different from past reports.[2,7,16–18] The present analysis of TCGA cases showed that the frequency of MMR gene mutation was 3% and 2% in *IDH* wild-type gliomas and *IDH* mutant gliomas, respectively. *MSH6* was the most frequently mutated MMR gene. *MSH6* was reported to be observed in recurrent gliomas treated with temozolomide, and the mutation was associated with temozolomide resistance.[2,7,16] In fact, MMR gene mutation was observed more frequently in recurrent gliomas than in primary gliomas in the present analysis. Comparing overall survival between MMR mutant and MMR wild type showed that MMR mutation was not a prognostic factor. *TP53* mutation was observed in most MMR gene mutant gliomas with *IDH* mutation, indicating that they were astrocytomas, and MMR gene mutation was rare in oligodendrogliomas. Expression analysis suggested that the TNF signaling pathway was upregulated, and the Rap1 signaling pathway was downregulated. Downregulation of the Rap1 signaling pathway was associated with migration and invasion of glioma cells.[19,20] The bone marrow metastases of glioblastoma observed in case 2 could be associated with downregulation of the pathway.

Conclusion

Two cases of MMR gene-deficient glioblastomas were reported, and their clinical course was considered to be associated with MMR gene deficiency based on the genetic characteristics of the tumors.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

All experiments using human samples were approved by the ethics committee of the Tokyo Metropolitan Komagome Hospital (No. 755, 2248).

Informed Consent

Written, informed consent was obtained from all patients involved.

Conflicts of Interest Disclosure

The authors declare that they have no competing interests.

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